

12

**EUROPEAN PATENT APPLICATION**

21 Application number: 86108615.5

51 Int. Cl.: **A 61 K 31/47**

22 Date of filing: 24.06.86

30 Priority: 27.06.85 IT 2132285

71 Applicant: **I.P.A. INTERNATIONAL PHARMACEUTICAL ASSOCIATED S.r.l.**, Via Casale Cavallari, 53, I-00156 Rome (IT)

43 Date of publication of application: 30.12.86  
 Bulletin 86/52

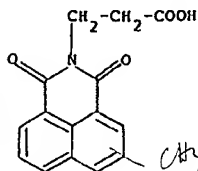
72 Inventor: **Malizia, Paolo**, Via Casale Cavallari, 53, I-00156 Rome (IT)

64 Designated Contracting States: **AT BE CH DE FR GB IT LI LU NL SE**

74 Representative: **Blanchetti, Giuseppe**, Studio Consulenza Brevettuale Via Rossini, 8, I-20122 Milan (IT)

54 **N-(2-carboxy)-ethyl-1,8-naphthalene imide and its salts for the treatment of diabetic retinopathies and neuropathies.**

57 Pharmaceutical compositions containing as the active principle N-(2-carboxy)-ethyl-1,8-naphthaleneimide of formula I



or non toxic salts thereof, are described.

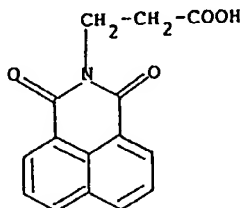
The compound I is endowed with antialdase-reductase and platelet antiaggregant activities.

**EP 0 206 322 A2**

PHARMACEUTICAL COMPOSITIONS AND COMPOUNDS FOR THE THERAPY  
OF DIABETIC RETINOPATHIES AND NEUROPATHIES

The present invention refers to pharmaceutical composition containing, as an active principle, therapeutically effective amounts of N-(2-carboxy)-ethyl-1,8-naphthaleneimide, of formula I

5



(I)

10

or a physiologically acceptable salt thereof, such as an alkali, earth-alkali, ammonium salt, salts with basic aminoacids such as lysine, ornithine and arginine, with primary, secondary or tertiary amines, with aminosugars, etc.

15

The lysine and N-methylglucamine are particularly preferred and provide a further object of the invention.

The compound I is endowed with inhibitory activity of the aldose-reductase, an enzyme responsible of the degradation of hexoses to polyols such as sorbitol and dulcitol.

20

In the presence of high hexoses levels, as it occurs for instance in the diabetes and in the galactosemias, an accumulation of said polyols occur in some tissues, and particularly in the eye, at the level of retina and crystalline, in the kidneys and in the peripheral nerves, with consequent onset of retinopathies, neuropathies,

25

- 2 -

thies and nephropathies which are the main complications of diabetes.

The compositions containing the compound I are therefore useful in human therapy for the treatment of 5 retinopathies, neuropathies and nephropathies on diabetic basis.

The compound I is known, having been described by El Naggar et al. in Egypt. J. Chem. 24 (2), 127-130 (1981) as endowed with antibacterial activity against Bacillus 10 strains. The compound I is also reported to have hypolipemic activity (J. Pharm. Sci. 1984, 73 (7), 956).

The U.S. Patent No. 3,821,383 discloses pharmaceutical compositions useful in the treatment of diabetes and 15 of its complications, containing as the active principle the N-(2-carboxy)-methyl-1,8-naphtaleneimide or 1,3-dioxo-1H-benzo[de]isoquinoline-2(3H) acetic acid. Said compound is therefore the lower homolog of the compound I which is however surprisingly more active and endowed with more advantageous pharmaco-therapeutics characteristics.

20 The compound I hereinbelow also referred to, for the sake of brevity, with the abbreviation IPA 955, or one of the salts thereof, may be administered according to the invention by the oral, parenteral or topical route, in form of pharmaceutical compositions containing pharmacolo- 25 gically acceptable excipients and optionally other active principles.

The topical route of administration is particularly important in the ophtalmic field because the etiological role played by aldose-reductase in the cataract with 30 darkening of the crystalline and in the retinopathies

- 3 -

induced by galactosemia and diabetes, is known. IPA 955 is therefore particularly useful for the topical administration in the eye in form of collyres comprising sterile solutions buffered to pH 7.2-7.4.

5           Suitable forms for the oral administration are capsules, tablets, sugar-coated tablets, syrups, solutions. Suitable forms for the parenteral administration are sterile vials or bottles containing the active principle, optionally mixed with a suitable carrier.

10           The dosage of IPA 955 will obviously depend on the seriousness of the pathology to be treated and on patient's weight and age; it may generally range, because of the limited toxicity of the compositions of the invention, from 1 to 100 mg/kg/die by the oral and parenteral route:

15           however, as a rule, lower dosages, from 1 to 50 mg/kg/die, will be generally sufficient. By the topical route it will be generally suited the instillation of 0.1-5% eye-washes (collyrium) 1-2 times a day.

            The unitary dosage forms for oral or parenteral  
20           administration may comprise from 10 to about 500 mg, administered from 1 to 4 times a day.

            The compound I is prepared from  $\beta$ -alanine and 1,8-naphtalic anhydride, according to the following example.

EXAMPLE 1

25           10 Grams (1 mole) of 1,8-naphtalic anhydride were added to a 98% solution of  $\beta$ -alanine (5.05 g, 1.1 mole) and sodium hydroxide (2.26 g) in 50 ml of water. After 6 hours at reflux, the mixture was cooled, acidified with diluted HCl and filtered. After washing with  $H_2O$  and double  
30           recrystallization from ethanol 95°, 6.42 g of IPA 955

- 4 -

were obtained (m.p. 319-321°C). 5 Grams of said product were dissolved in absolute ethanol, added with the stoichiometric amount of sodium ethoxide in absolute ethanol, were left overnight to react at the room temperature. The 5 precipitated sodium salt is then filtered, washed with absolute methanol and dried at 60°C.

NMR Spectrum ( $\delta$ ;  $\text{CDCl}_3/\text{DMSO}$  80/20):

2.43-2.85 (2H, t); 4.10-4.55 (2H, t); 7.45-7.90 (2H, m); 8.00-8.60 (4H, m).

10 EXAMPLE 2

1.1 Grams of L-lysine were added to a solution of 2 g of IPA 955, prepared according to the Example 1, in 25 ml of 95% ethanol, at room temperature and under stirring. After 4 hours, the mixture was cooled to 0-5°C and the 15 precipitated salt filtered, whose chemico-physical characteristics agree with those expected for the captioned product.

The N-methylglucamine salt was similarly prepared, by using, instead of lysine, 1.5 g of methylglucamine.

20 The acute toxicity of IPA 955, its aldose-reductase inhibiting activity in comparison with quercitrin and its antiaggregant activity in comparison with ticlopidine have been determined.

Acute toxicity

25 The acute toxicity of IPA 955 has been studied in the mouse after oral and intravenous administration. The  $\text{LD}_{50}$  values were calculated according to the Litchfield and Wilcoxon method (J. Pharm. Exp. Therap. 1949, 96, 99).

IPA 955, at the tested doses, proved to be practically 30 devoid of toxicity.

- 5 -

Aldose-reductase inhibitory activity

Crystallins of adult male rats have been homogenized in 0.1 M phosphate buffer pH 6.2. The supernatant, after centrifugation at 3000 x g for 15 minutes, has been used.

5 The supernatant contained 13 mg/ml of proteins. Quercitrin (Fluka, Buchs) 1 mM dissolved in DMSO: H<sub>2</sub>O (1:10) has been used.

The aldose-reductase activity has been tested by the method described by S.D. Varma and J.H. Kinoshita

10 (Biochem. Pharmacol. 25, 2505, 1976) using 1 ml of final volume containing NADPH (20  $\mu$ l of a 10 mM solution), DL glyceraldehyde  $1.5 \times 10^{-3}$  M final, LiCl<sub>2</sub> (25  $\mu$ l of a 1 M solution) and 150  $\mu$ l of a supernatant. The blank contained the same substances, except the supernatant. As a reference

15 drug, quercitrin, a known aldose-reductase inhibitor, has been used.

IPA 955, with a K<sub>i</sub> of 1.04  $\mu$ M, proved to have an activity about 6 times higher than that of quercitrin (K<sub>i</sub> = 6.00  $\mu$ M).

20 "In vitro" platelet antiaggregant activity

The platelet antiaggregant activity of IPA 955 has been determined in comparison with a known antiaggregant activity, Ticlopidine, on the platelets of rabbit blood added with 3.6% sodium citrate in an amount of 10%. The

25 blood was centrifuged for 15' at 1200 rpm and the supernatant was recovered for its platelet content. The residue has been further centrifuged with the aim of obtaining a "poor" plasma (3000 - 3500 rpm). The plasma platelets obtained after the first centrifugation were suitably

30 diluted with the poor plasma, counted and the rich plasma

- 6 -

(first centrifugation) was diluted according to the platelet count so as to obtain a final concentration of platelets of  $25 \cdot 10^4 \div 30 \cdot 10^4 / \text{mm}^3$ .

The aggregation was induced by the addition of ADP 5 and collagene at the specified concentrations.

The results are reported in the following Tables.

ADP-INDUCED AGGREGATION					
Percent inhibition					
Compounds	Dose	conc. 5.0 $\mu$ M	conc. 6.25 $\mu$ M	conc. 8.3 $\mu$ M	conc. 12.5 $\mu$ M
IPA 955	0.625 mM		8.4%		23.17%
TICLOPIDINE			9.7%		15.66%
IPA 955	1.25 mM		14.6%	16.3%	16.85%
TICLOPIDINE			24.4%	19.75%	20.02%



AGGREGATION BY COLLAGENE			
Compounds	Dose	Percent inhibition	
		25 µg/ml	50 µg/ml
IPA 955	0.625 mM	41.05%	
TICLOPIDINE		45.26%	
IPA 955	1.25 mM	48.83%	38.66%
TICLOPIDINE		66.5%	59.6%

- 9 -

According to the values reported in the Tables, IPA 955 turns out to be endowed with an high platelet antiaggregation activity, ranging from 40 to 50% in the case of collagene induced aggregation; in the ADP-induced 5 aggregation the percent inhibition is about 20%; in both cases practically comparable, even if slightly lower than that of Ticlopidine.

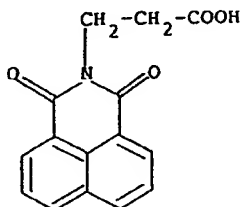
- 10 -

CLAIMS for the Contracting States:

BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Pharmaceutical compositions for the therapy of  
 5 diabetic neuropathies, retinopathies and nephropathies  
 containing as the active principle N-(2-carboxy)-ethyl-  
 1,8-naphtaleneimide of formula I

10



(I)

- or its non toxic, physiologically acceptable salts.
- 15 2. Compositions according to claim 1, containing as  
 the active principle the lysine or N-methylglucamine salt  
 of compound I.
3. Compositions according to claim 1 or 2 for the  
 oral, parenteral or topical administration in form of  
 20 capsules, tablets, sugar-coated tablets, syrups, solu-  
 tions, sterile eye washes, collyriums, vials.
4. Compositions according to claims 1, 2 or 3 contain-  
 ing from 10 to 500 mg of the compound I or the equivalent  
 of the salt thereof for the oral or parenteral forms and  
 25 from 0.1 to 5% of active principle for the topical forms.
5. N-(2-Carboxy)-ethyl-1,8-naphtaleneimide lysine  
 salt.
6. N-(2-Carboxy)-ethyl-1,8-naphtaleneimide N-methyl-  
 glucamine salt.
- 30 7. Use of N-(2-carboxy)-ethyl-1,8-naphtaleneimide or

**0 206 322**

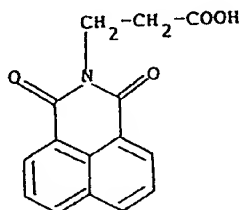
- 11 -

of a non toxic salt thereof for the preparation of a medicament useful for the therapeutic or prophylactic treatment of diabetes and of complications thereof.

- 19 -

CLAIMS for AT

1. Process for the preparation of pharmaceutical compositions for the therapy of diabetic neuropathies, retinopathies and nephropathies characterized by admixing N-(2-carboxy)-ethyl-1,8-naphtaleneimide of formula I



(I)

10

with suitable, non toxic excipients.

- 15 2. Process for the preparation of N-(2-carboxy)-ethyl-1,8-naphtaleneimide lysine or N-methylglucamine salt characterized in that N-(2-carboxy)-ethyl-1,8-naphtaleneimide is reacted with substantially equimolar amounts of L-lysine or N-methylglucamine in the presence of a solvent.
- 20 vent.